Allogeneic haematopoietic stem cell donation and transplantation across the MHC class I barrier: "Faster is better than more. More is better than less".

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General Introduction

The Aztec god Quetzalquatl or Feathered Snake

On the concept of self and non-self

In the year 1519 AD the Spanish conquistador Hernando Cortez conquered the Aztec empire. The circumstances were very much in favour of Cortez. According to tradition, the Aztecs mistook the arrival of Cortez and his army for the arrival of their popular god Quetzalquatl (Feathered Snake). According to Aztec belief Quetzalquatl was a patron god, a benefactor, who in contrast to others did not asked for human sacrifices. Centuries before these event he supposedly had fled the Aztec empire on a raft made out of snakes, after being tricked by his opponent Tezcatlipoca (Smoking Mirror). According to prophecy, he would return someday to reclaim the kingdom and restore his law and order. The Aztec calendar predicted his return on several dates. One of these corresponded to 1519 AD. Cortez's ships and impressive army equipped with horses, guns and canons, could only be perceived by the Aztecs as the supernatural attributes to be expected from an almighty god like Quetzalquatl. This prevented the Aztecs from an immediate attack on the intruders. It was therefore easy for Cortez to prepare his army and enter the kingdom. King Montezuma even welcomed them personally. Cortez and his men on the other hand perceived the Aztecs as heretics or non-believers practicing bloody human sacrifices. In their eyes the empire had to be conquered and its people converted. It thus happened that the invited guests (with some help of neighbouring tribes) overthrew the reign of the Aztec king with relative ease. One could not think of a more sinister ending for the Aztecs to their legend of the return of the patron god Quetzalquatl.

This is a good example of the concept of self versus non-self. It is important to keep in mind is that this is not merely a metaphysical concept (the question of what *is* self and what *is* non-self). It is epistemological problem, which addresses how to discriminate between self and non-self. Is non-self recognition based on sensing a foreign fingerprint or rather the absence of the self-fingerprint? Furthermore, in accordance with Kants '*Kritik der reinen Vernunft*', recognition is restricted to the limitations of the senses, the frame of references and faculty of cognition. Certain features therefore become irrelevant, as the beholder does not notice them. Because of these limitations it is possible to mistake non-self for self and vice versa. Just like Montezuma who believed the Spanish army to be an attribute of the Aztec pantheon because of their appearance and his notion of the world. The Spaniards did not resemble any group of people they had encountered before. The thought of the Spaniards as human beings or an invading foreign army was from his point of view highly unlikely. He probably did not question their intentions, something he would have done if he knew that these were mere humans. The Spaniards clearly had a different point of view and perceived the Aztecs as very different but surely human.

There is a parallel between these past events and unrelated haematopoietic stem cell transplantation (SCT), the latter being the main topic of this thesis. Unrelated SCT concerns two distinct individuals: the donor and the recipient; the recipient being a patient suffering from a haematological disorder. If we compare SCT to the Spanish conquest of the Aztecs,

than Spain would take the place of the donor, Cortez's army the graft and the Aztec empire the recipient. Both parties are able to decide between self and non-self. The outcome of this decision will determine, for a large part, transplantation outcome.

The human immune system

The system most predominantly involved in self and non-self recognition after transplantation is the immune system. Throughout life, all human beings depend on their immune system in defending themselves against potentially life- threatening infections by a variety of agents like viruses, bacteria, and uni-cellular or multi-cellular eukaryotes. Besides defence against pathogen, the immune system also clears out damaged or cancerous cells and tissues. Ironically enough, the immune system of either donor or recipient can hamper good SCT outcome by responding to foreign cells (alloreactive immune response) as if they were potentially life- threatening invaders.

The human immune system has a vast arsenal of weaponry generally divided into those involved in the innate and in the acquired immune response. The first concerns physical barriers like the skin and the mucosal epithelia of the respiratory, gastrointestinal and reproductive tracts, the complement system, and phagocytic cells and natural killer (NK) cells. The latter response is antigen specific and can establish memory, which causes a strong and rapid immune response against a secondary infection by the same pathogen.

The acquired immune system involves the cellular and humoral response. The cellular response comprises T cells recognising foreign antigens with their T cell receptors (TCR) in the context of the major histocompatibility complex (MHC) on antigen presenting cells (APC). There are several types of T cells. The CD8+ T cells generally have the cytotoxic activity. CD4+ T cells generally function as helper or regulatory cells involved in activation and clonal expansion of lymphocytes, or deactivation and tolerance against certain antigens. The humoral response involves B cells producing antibodies and can commence independent of MHC.

Recognition of foreign (allogeneic) antigen, like an ABO blood type difference, foreign MHC, and minor histocompatibility antigens of the donor or recipient can mediate a very aggressive immune response against foreign cells and tissues. Cell types of both innate and acquired immune can take part in this. A recipient can accept the graft if the immune system does not recognise donor antigens as non-self or if recognition does not lead to a detrimental immune reaction. The same goes for the donor lymphocytes present in or derived from the stem cell graft, which potentially can react against foreign MHC antigens of the recipient leading to graft versus host disease. An allogeneic immune response mediated by these donor derived lymphocytes recognising non-self antigens can result in transplant related complications and thus poor transplantation outcome or even death of the recipient.

Major Histocompatibility Complex

The genes encoding for the most important antigens involved in transplant related immune response are clustered within the Major Histocompatibility Complex. Human MHC is named human leukocyte antigens (HLA). Until now six human HLA genes on the short arm of chromosome 6 have been described to be important for SCT outcome, namely HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DQB1 and HLA-DPB1.[1,2] HLA antigens can be serologically typed as broad or split antigens. Broad antigens are those antigens, which can be subdivided by serological techniques into two or more so-called split antigens, e.g. HLA-B5 contains the splits HLA-B51 and HLA-B52. Several diffent HLA alleles can encode for the same split antigen. HLA can be typed on allele level, 'high resolution typing'. using following techniques: the polymerase chain reaction sequence specific oligo (PCR-SSO), polymerase chain reaction sequence specific primer (PCR-SSP) and sequence based typing (SBT) of which the last is especially important for part of the HLA-C alleles.

By means of structural and functional differences, and differential expression, HLA genes can be split into two classes. HLA-A, HLA-B and HLA-C genes are part of the MHC class I cluster and are expressed by all nucleated cells and platelets. HLA-DRB1, HLA-DQB1 and HLA-DPB1 genes are part of MHC class II and are expressed by a limited group of cell types also referred to as professional APC, such as dendritic cells, CD4+ T cells, B cells and monocytes.

MHC class I molecules consists of a membrane anchored heavy chain of 44 kD and a non-covalently associated light chain of 12 kD called β2-microglobulin (encoded for on chromosome 15) that is required for the complex stability.[3-5] The extra cellular part of the heavy chain comprises the α1, α2 and α3 domain (figure 1a).[6] The α1 and α2 domains form two α helices supported by an eight-stranded β sheet that functions as the peptide-binding groove (figure 1b).[7] An antigen peptide, either self or foreign, binds on the β sheet between the two α helices. A peptide-MHC complex (pMHC) is formed. The peptide-binding groove of the MHC class I is closed at both ends resulting a size restriction of the bound peptides (8-13 amino acids).[8-10] These peptides are generally derived from endogenously synthesized proteins, like viral antigens or self proteins, which have been degraded by the proteasome.[11-13]

The overall structure of the MHC class II molecule resembles to that of class I. The molecule consists of a heavy chain (α) of 33 kD and a light chain (β) of 28 kD.[14] In contrast to MHC class I both chains are membrane bound and contain two extra cellular globular domains (α1, α2, β1 and β2). The conformation of the α2 and β2 domains is comparable to that of the α3 and β_2-microglobulin of class I. The α1 and β1 domain also form two α helices supported by a β sheet that functions as a peptide-binding groove.[15] In contrast to class I, the MHC class II peptide binding groove is open at both ends, resulting in the binding of longer antigen peptides (13-25 amino acids).[16-18] The peptides presented by MHC class II molecules are generally

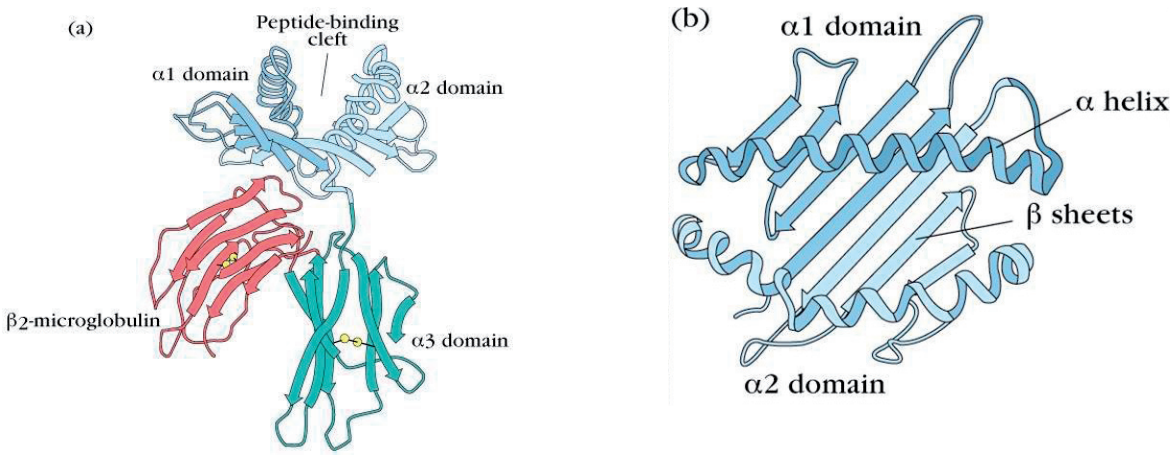

Figure 1. Ribbon diagram of the extra cellular domains of HLA-A2 (a) and a top-view of the peptide binding groove in HLA-A2 as seen by the T cell receptor (b)*. (adapted from Bjorkman et al. Nature 329:506, 1987)*

derived from extra cellular proteins which were taken up in endosomes or lysosomes.[19,20] The MHC loci are highly polymorphic. The HLA-A, -B, -C, -DRB1, -DQB1 and DPB1 genes show a great deal of variation between individuals, although some loci more than the others.[21] Each locus consists of two co-dominant alleles. Conversely, it is common to have two different alleles per gene and to express both. Although MHC is highly polymorphic, MHC molecules have both polymorphic and constant or conserved sites. The CD4 molecule has binding affinity to such a conserved motif in MHC class II molecules while the CD8 molecule has affinity for such a motif shared by MHC class I molecules. This results in CD4+ T cells being restricted to antigen recognition in the context of MHC class II and CD8+ T cells being restricted to MHC class I.

The majority of polymorphic residues of the MHC molecules are located in or near the peptide-binding groove (although the β1 domain of class II is a lot more polymorphic than the α1 domain). The amino acid differences can be located within the TCR binding sites and thus influence the affinity to particular TCR. Amino acid differences in regions important for peptide binding provides MHC molecules with alternate peptide-binding properties influencing antigen conformation within the peptide binding groove or which antigen is presented. Both can result in different TCR-MHC binding properties and recognition. Still matters are more complicated than presented here as all differences can change the quaternary structure of the MHC peptide complex. In theory, amino acid changes in one region can affect the structure or function of other regions. Some amino acid differences however, do not lead to a structural or functional difference due to structural similarity or their position within MHC.

In addition to the HLA molecules previously mentioned there are also three “non-classical” MHC class I molecules, namely HLA-E, -F, and –G. These appear to have only limited polymorphism and expression, and appear to play a role in mediating NK cell responses and reproductive processes.[1]

MHC-TCR interaction and T cell activation

Specific recognition of a particular peptide or peptide in the context of MHC is accomplished by a membrane bound T cell receptor (TCR) (Figure). Strong TCR-MHC binding results into T cell reactivity. The TCR complex consists of highly diverse αβ or γδ heterodimers and the γ, δ, ε and ζ chains of the invariant accessory protein CD3.[22,23] T cells express the αβ TCR are predominant. The α and β chains participate in the interaction MHC peptide complex and the CD3 chain initiates the intracellular signalling after triggering of the TCR.[24-26] The α chain and β chain include a constant domain and a variable domain.[27] The variable domain is generated during T cell development by rearrangement of variable (V), diversity (D), joining (J) and constant (C) gene segments, forming the antigen specific binding site of the TCR. [28] Recombination of the V, D and J gene segments results in a TCR repertoire with great diversity. Regions within the V region encode for hypervariable amino acid sequences, called complementary determining regions (CDR) found on both α and β chains. They serve as primary contact points with the α helices of MHC (CDR1 and CDR2) and the peptide presented by MHC (CDR3).[28,29]

During T cell development, thymocytes generate a vast array of clonally expressed αβ TCR that mediate the recognition of foreign peptides in the context of self-MHC. Prior to selection the TCR repertoire has an extensive potential diversity up to ~10^{14} different structures.[28] Selection processes in the thymus and peripheral lymphoid organs, however, shape the functional TCR repertoire. T cell must bind to self-pMHC with sufficient affinity for positive selection and peripheral survival. If these affinities are too high, the T cells will be deleted by negative selection, a process that has been estimated to operate on 20-30% of the thymocytes.[30-32] The window of binding affinities that separate positive and negative selection appears to be very narrow, differing by as little as threefold.[33] In principle, this means that individual TCR repertoires are based on self-pMHC (the frame of reference), resulting in an inimitable and unique TCR repertoire. This is a primary cause for the unpredictability of T cell mediated alloreactivity against allogeneic MHC molecules.

Wu and colleagues proposed a two-step mechanism TCR binding to pMHC based on their results.[34] They uncovered that mutations in the α helices changed initial association between TCR and MHC while mutations in the β plated sheet changed TCR-pMHC complex stability. They hypothesised that first an initial association between the TCR CDR1 and CDR2 loops and α helices takes place. If this initial association is strong enough and lasts long enough, than the CDR3 loops can fold and make contact to the peptide bound by MHC. T cell activation will only take place on the formation of stable peptide contacts. This model suggests that

TCR dock onto pMHC independent of the peptide. It is intriguing that the CDR3 loops of the TCR are highly flexible and mobile in the unbound state and adopt conformations that cannot specially contact peptide residues without substantial rearrangements, sometimes up to 15Å. [35-37] In contrast the CDR1 and CDR2 loops are much more rigid and generally show little or no rearrangements when binding peptide- MHC. Thus the TCR may scan MHC molecules using a lock and key type of binding with its CDR1 and CDR2 loops, followed by an induced fit of its CDR3 loops over the peptide.[38,39] A two-step mechanism might be necessary to scan the many thousands of peptides displayed by MHC molecules on the antigen-presenting cell. It would also help to explain the inherent cross reactivity of TCR, because the CDR3 loops of TCR could adopt several conformations and thus form stable contacts with structurally very different peptides.[40] This model can also have implications for thymic selection of T cells during which T cells are skewed to recognise self-MHC alleles. Thymic selection may imprint MHC restriction by enriching for TCR that can scan a particular self-MHC and may be unable to scan other MHC efficiently.
Besides the MHC peptide complex and TCR interaction, the activation and clonal expansion of T cells also involves costimulatory signals provided by accessory molecules. There are quite a few costimulatory molecules. Many are broadly distributed while others are restricted to specific cell types. T cells not receiving these costimulatory signals can go into a nonresponsive state or die by apoptosis.[41,42] Blockers of these costimulatory signals can have immunosuppressive properties and have shown to lead to prolonged allograft survival.[43-45] Many immunosuppressive drugs however are unspecific leaving patients defenceless against pathogens or possible tumors and secondly have an endless list of side effects.

Direct and indirect allorecognition

Lymphocytes recognise allogeneic MHC-peptide complexes on the surface of foreign cells because different MHC molecules associate differently with its TCR than self-MHC. Allogeneic MHC can trigger alloreactivity directly by its structural and functional differences or altered peptide presentation. Besides direct allorecognition of the whole quaternary structure of allogeneic pMHC there is also an indirect pathway.[46-49] This pathway involves APC presenting peptides of allogeneic origin (for instance MHC) in context of self-MHC. These peptides are derived from MHC molecules shed from foreign cells, taken up by APC and processed and presented on their cell surface by MHC molecules, mainly MHC class II recognised by CD4+ T cells.[50] The frequency of T cells taking part in the direct recognition of MHC molecules is about 100 fold higher than that of T cells involved in the indirect recognition pathway.[51] There are two different explanatory hypotheses. The multiple binary complex hypothesis assumes that T cells recognise a specific foreign peptide in the context of a certain MHC molecule. Because a single foreign MHC molecule can bind a fast array of antigen peptides, it forms several hundred distinct MHC-peptide complexes each recognised

by a distinct clone of alloreactive T cells. The high determinant density hypothesis on the other hand assumes alloreactive T cells to be specific for the foreign MHC molecule with little or no specificity for the bound peptide. In this case all MHC molecules of any given isotype can serve as ligands for the alloreactive T cell, thereby creating a very high ligand density and a high precursor frequency.[52,53]

Besides recognition of foreign MHC, the innate immune system is also able to detect the absence of self-MHC. The cell type responsible for this kind of allorecognition is the NK cell. NK cells are normally inhibited by specific inhibitory KIR ligands.[54] HLA-C molecules bearing the Ser77 and Asn80 motif (C1) are ligands for the inhibitory KIR2DL2 and KIR2DL3 while HLA-C molecules bearing the Asn77 and Lys80 motif (C2) are ligands for the inhibitory KIR2DL1.[55,56] Besides HLA-C, HLA-A and –B also have inhibitory KIR ligands. NK cell mediated alloreactivity can occur when target cells do not have the same inhibitory motifs as the responder.[57]

The clinical practise of haematopoietic stem cell transplantation.

Stem cell transplantation is the treatment of choice for a variety of haematological disorders such as leukaemia's and bone marrow failure syndromes. Before the actual transplantation, the patients receive a sub-lethal dose of chemotherapy and in numerous cases total body irradiation to eradicate their own haematopoietic system including their own haematopoietic stem cells and lymphocytes. This eradication is not 100%, as that would require lethal doses. This treatment makes the patient quite immune deficient and most cases the patient is placed in an ultra clean room or laminar airflow system to avoid pathogenic infections. Unfortunately viral, bacterial, fungal, infections still occur, sometimes with fatal consequences. Even infections that pose no serious threat for healthy individuals can be lethal in this situation. This is just one of the transplant related hazards a patient has to face.

The haematopoietic stem cells are infused intravenously and will restore the haematopoietic system of the patient if not rejected by the patients remaining immune cells. A major complication is graft versus host disease (GVHD). This disease is a result of donor derived immune competent cells mounting a response against tissue antigens of the patient, such as the HLA antigens. The main targets for the alloimmune reaction are the patient's skin, liver and gut. Acute GVHD develops within the first 100 days after transplantation. Acute GVHD is divided in four grades of severity of which the first grade is ambiguous and the fourth most severe and often lethal.[58] GVHD that develops after these 100 days is called chronic GVHD and is subdivided in limited and extensive.[59,60] GVHD incidence and severity can be reduced by T cell depletion of the graft. This can be accomplished by positive selection of stem cells (CD34+) or by the removal of T lymphocytes by immunological or physical methods, for example by T cell specific antibodies or counterflow centrifugation.[61,62] Depleting T cells, however, increases the occurrence of graft rejection by the patient and disease relapse. The

latter shows that a graft versus leukaemia effect often accompanies GVHD. Treating the patient with a donor lymphocyte infusion after a disease relapse can induce this graft versus leukaemia effect. It can however also induce GVHD. This all shows that the treatment after SCT is balancing between the chance on a disease relapse and the chance on GVHD. Thus, there are many transplant related hazards for a patient undergoing SCT plus the chance of disease reoccurrence. Both can be lethal. Furthermore, the immune compromised status of the patient plus the use of immune suppression to avoid rejection and GVHD increases the susceptibility to infections; another transplant related hazard. It is therefore that in most cases recipients of a HLA identical graft have a superior chance on survival.
Because of all this, there is the convention in SCT research to divide patient mortality after SCT into transplant related mortality and relapse related mortality, as they are opposites. Recipients of an HLA identical graft have a higher chance of disease relapse related death than on transplant related mortality opposed to recipients of an HLA mismatched graft.

From HLA to stem cell donor registries and a world wide network

It is of the utmost importance to identify the risk of GVHD before transplantation and to search for the most compatible donor (based on MHC matching and in vitro histocompatibility testing). Unfortunately, approximately 70% of eligible patients lack a MHC identical sibling donor. Members of the extended family or voluntary unrelated donors are feasible alternatives if donor and recipient are MHC matched or have acceptable MHC differences that do not preclude successful SCT. An unrelated stem cell donor search depends on a pool of voluntary up front tissue typed donors.
During the seventies and eighties the idea of unrelated up front HLA typed stem cell donors formed. The Deutsche Gesellschaft für Bluttransfusion meeting in 1971 inspired the idea of a European organisation for the recruitment of HLA typed donors from the pool of blood transfusion donors.[63] This proposed organisation, entitled Europdonor Foundation, was never put into effect, but instead developed in the Dutch donor registry, which was officially founded in 1988 by Jon van Rood. This foundation still bares the same name. The initiative to institute national registries started in 1974, when Shirley Nolan founded the Anthony Nolan Trust in the UK; originally to recruit Stem cell donors for her son. Many countries followed this initiative and founded a national donor registry. The New York Blood Centre introduced another phenomenon, the public cord blood bank, in 1991. During the late eighties and early nineties umbilical cord bloods were discovered to contain enough haematopoietic stem cells to lead to a successful transplantation. First only used in sibling transplantation they were later also used for unrelated SCT. One of the advantages of cord blood derived stem cells above bone marrow derived stem cells is that they are less immunogenous and are transplanted successfully over a greater HLA mismatch barrier. On the other hand, in most cases they do not include a sufficient number of cells for recipient weighing more than

40 kg. This because the number of stem cells needed for a successful engraftment correlates with the body mass of the recipient and the limited amount of stem cells within a single cord blood unit.
Al these local initiatives provided a large stem cell donor pool, however there was a high chance that the only acceptable donor for a patient was registered and living abroad. The problem of searching all registries separately resulted in a large amount of unproductive work (there may be no donor) at sizeable expense and with considerable time delay. In 1989, the first edition of Bone Marrow Donors Worldwide (BMDW) was published to make international cooperation between registries more easy. BMDW, founded by Jon van Rood, was at first a collection of paper listings of the HLA phenotypes of bone marrow donors of the eight participating registries. From 1989 and onwards, important progress has been made. First BMDW has evolved from a paper listing to a database, accessible via the Internet and accompanied by a donor-matching program. Secondly, the number of participating registries has increased considerably and is still increasing. In 2004, BMDW integrated 54 registries from 39 countries, accompanied by 37 cord blood registries from 21 countries. This led to an enormous increase of available up front typed donors and thus available HLA phenotypes. At the end of 2005 (November 16th), BMDW included ten million donors. Subsequently the participating Cord blood registries contributed 189,331 cord blood units. In addition, the resolution of HLA typing increased tremendously. At first HLA-A and HLA-B broad antigens were typed. In the early nineties, HLA-A, -B, DR split antigens became the standard for tissue typing. From 1996 and onwards high resolution allele typing was routinely used to determine HLA-A*, -B*, -C*, DRB1* and HLA-DQB1* loci of the patients and the preliminary selected donors. In the future, these techniques could also be used for up front typed donor pool, however this is still in its early stages.

The allogeneic donor search as preformed by the Europdonor Foundation
In short I will here describe the outline for the allogeneic donor search process for a patient who lacks an HLA (genotypically) identical sibling or other related donors. The patients are typed for HLA-A, -B, -C, -DRB1/3/4/5, -DQB1, -DPB1 at allele level. After a patient is reported to Europdonor, a search is started by looking in BMDW for an HLA-A, -B, -DR broad- or if possible split-identical donor and in consultation with the transplant centres a selection of donors and or cord blood units is made. If none is found, a five out of six antigen matched donor is looked for. Donors are selected on the highest chance of being 12/12 matched with the patient. In order to do so the following items are taken into account: the level of typing of the donors present, the linkage disequilibrium, the country of donors and are available the ethnic origin of donors. The number of HLA-A, -B, -DR split typed donors requested for high resolution HLA typing are based on the frequency of the patients HLA phenotype in BMDW, the presence of rare alleles, and the urgency of the search (medium:

5, range: 3 – 9). If possible, a higher number of potential donors are requested for patients with a less common HLA phenotype and/or high transplantation urgency. If the patient appears to have a frequent HLA haplotype or in case of consanguinity or other interfamilial relationships, an extended family search is started simultaneously. In most cases of finding both, an acceptable family donor and an acceptable unrelated donor the family donor is chosen.
In the period 1987-1992 a 6 out of 6 or 5/6 matched donor (HLA-A, -B, -DR on split level by serology) was selected for both the adult and paediatric patients. Between 1992 until 1996 an 8/8 or 7/8 matched donor (HLA-A and –B on split level by serology and HLA-DRB1* and –DQB1* on allele level) was selected. From 1996 and onwards a 10/10 or 9/10 matched donor (HLA-A*, -B*, -C*, -DRB1*, -DQB1* on allele level) is selected. Additional HLA-DPB1 matching is favoured if possible but not requered. In all allogeneic donor searches, a mixed lymphocyte culture is performed routinely and from 1992 onwards the cytotoxic T lymphocyte precursor assay is also being performed when possible. This is done to locate possible HLA mismatches that were not detected by serology, new alleles not detected by DNA typing and acceptable mismatches in case of mismatched donors. The outcome of these assays is predominantly used to choose the best donor among a group of donors and to determine whether or not to T cell deplete in case of paediatric patients. Donors are seldom excluded from transplantation because of the outcome of the CTLp assay if no there is no alternative donor. After this extensive histocompatibility testing, the most suitable donor is selected. Moreover, if possible a backup donor is selected, which is used in case the best donor failed to donate (third chapter in this thesis). Next the work up of the chosen donor is started.
If no ≥ 9/10 matched donor or 6/6 matched cord blood unit is available, transplantation with a 8/10 matched or a haplotype mismatched family donor or a mismatched cord blood unit may be considered. The choice between these two options depends on the diagnosis of the patient and the preference of the transplant centre. Adult patients are less likely to be transplanted with a cord blood unit, due to the limited amount of cells within single cord blood units.

HLA phenotype distribution within the human species.
Patients do not have equal chances on an unrelated donor because of the HLA phenotype distribution. The HLA loci are located on the short arm of chromosome 6 and therefore inherited as haplotypes. A haplotype is defined as ‘half of a genotype’ of which all genes are located on the same chromosome. MHC normally passes on to the next generation with two possible sets of alleles including one allele per gene. Sporadically recombination within the MHC occurs, which results in crossover haplotypes. Tight linkage between the HLA loci and the effect of selective forces caused many haplotype combinations to have higher

frequencies than the frequency expected from the Hardy-Weinberg equilibrium (a 'random distribution'). This is 'linkage disequilibrium'.
Without linkage disequilibrium the sheer number of different alleles could have led to over 10^{26} combinations. Linkage disequilibrium therefore increases stem cell donor availability because common haplotypes and phenotypes give a higher chance on a matched donor for many patients than expected in case of Hardy-Weinberg equilibrium.
However, many haplotypes are rare, which makes it difficult to provide every patient with a donor. Haplotype frequencies differ between populations. For example the HLA-A1, -B8 and -DR17 haplotype is very common in the Netherlands and occurs in around seven percent of the population.[64] In China, for instance, this haplotype is very infrequent (www.BMDW.org). The chance of finding a donor for patients originating from under-developed countries is a lot smaller than for patients originating from developed countries. Most of the countries participating in BMDW are developed. Regions with more than 100,000 donors include many Western European and several Mediterranean countries, the U.S.A., Canada, Australia, Taiwan, and Japan. Most under-developed countries are either not represented or in some cases contribute relatively small stem cell donor populations. This leads to a predomination of certain populations within the donor pool and an under representation of other groups. Patients originating from underdeveloped countries therefore cannot benefit from the large donor pool. A problem also faced by patients who are from mixed origin. Due to differential HLA haplotype distribution between populations, it is extremely difficult to find a HLA matched unrelated donor in either of the populations of ethnic origin.

Acceptable mismatches
In the Netherlands, fully HLA-A, -B, -C, -DRB1, -DQB1 matched unrelated stem cell donors are available for over 60% of Caucasoid patients and only 15% of patients from other origins. These donor search results and similar results published by others illustrate the need of a tool to define acceptable or permissible HLA differences.[65-67]
In clinical practice, some patients with major HLA mismatches have had successful transplantation outcome. Maruya and colleagues identified specific donor/recipient HLA mismatches that did not elicit a strong immune response within a large cohort of transplantations of single HLA-A, -B or -DR antigen mismatched living-donor kidneys.[68] They defined permissible mismatches as those resulting in more than 85% graft survival after three years. The results were later confirmed by van Rood and colleagues after analysis of the Eurotransplant database.[69] On the other hand Doxiadis and colleagues demonstrated that some HLA mismatches were associated with a significanty increased graft loss and named those taboo mismatches.[70,71]
Several attempts have been made to find acceptable mismatches within stem cell transplantation. The question addressed was which polymorphic regions and which amino

acid substitutions within MHC are important for allorecognition and which are not. Both Ferrara and Oudshoorn proposed that amino acid substitutions on certain positions in the peptide binding groove of MHC class I lead to increased T cell alloreactivity.[72,73] These positions were respectively 114 and 116, and 97, 99, 101, 114 and 116. Older studies also proposed position 156 on one of the α helices to be important.[74]

Other groups did not emphasise certain positions but took the whole structure of MHC molecule or at least the α1 and α2 domain of MHC class I or α1 and β1 domain of class II into consideration. This lead to two algorithms, HistoCheck and HLAMatchmaker, which have been put forward to be able to differentiate between acceptable and non-acceptable MHC differences. Both algorithms estimate the degree of MHC dissimilarity between donor and recipient, but use different strategies. HistoCheck uses structural data on MHC molecules and functional similarity of amino acids to calculate a sequence similarity matching (SSM) score.[75] The emphasis lies on amino acid positions involved in MHC peptide binding or those important for recognition by T cell receptors. High SSM scores correspond to high dissimilarity between MHC molecules and presumably should correlate with strong T cell alloreactivity. The second algorithm, HLAMatchmaker, based on the humoral response, only includes amino acid sequences accessible for alloantibodies. Subsequently, it converts each MHC class I allele into a linear string of amino acid triplets, and then determines, by intralocus and interlocus comparison, which allogeneic amino acid triplets on the MHC molecules are not shared with the recipient. [76] High numbers of mismatched triplets correlate with induction of alloantibodies after kidney transplantation.[77,78] However, it is very probable that HLAMatchmaker, which is based on B cell mediated alloreactivity, cannot predict SCT outcome, where T cells are the key players in the alloreactive immune response after SCT. T cell and B cell have different ways to recognise allogeneic MHC class I. While the antibodies connect to and directly recognize small epitopes in MHC, T cells depend on the entire quaternary structure of pMHC. Petersdorf and colleagues made a distinction between allele and antigen mismatches.[79,80] Alleles encoding MHC molecules that are being recognized by alloantibodies induced by pregnancy or blood transfusions have been identified as MHC antigens. By convention in stem cell transplantation outcome analysis, the term MHC allele mismatch refers to a donor and recipient both having a different MHC allele out of a family that encodes for the same MHC antigen.[79] Their results were that an antigen mismatch leads to a stronger allogeneic immune response in haematopoietic stem cell transplantation than an allele mismatch. To summarize the general idea on acceptable and taboo mismatches: More amino acid differences between MHC will lead to a stronger allogeneic immune response, and certain amino acid differences are more immunogenic than others. To uncover acceptable mismatches is one of the key factors to increase the potential donor pool for patients lacking a fully MHC matched donors.

Short summary of what to expect in the coming chapters (aim of the thesis)
This thesis covers two distinct but related topics. **Chapter 2 and 3** addresses possible strategies for increasing efficiency and effectivity of the unrelated stem cell donor search. **Chapter 2** addresses the present bottlenecks that hamper donor search success and leave patients without a transplant on the basis of an analysis on donor searches performed for Dutch stem cell transplantation by the Europdonor Foundation. **Chapter 3** concerns the relevance of identifying a back-up donor after choosing the most compatible donor.
Secondly, this thesis addresses cytotoxic T cell-mediated alloreactivity against foreign MHC class I, which in general causes graft versus host disease and transplant related mortality after haematopoietic stem cell transplantation. In vitro T cell alloreactivity is routinely measured in our clinic before transplantation by the use of a cytotoxic T lymphocyte precursor (CTLp) assay to select the most compatible donor. In order to uncover which MHC amino acid sequence differences are important for T cell mediated alloreactivity the analyses described in these chapters were performed on donor-recipient pairs with a single MHC class I mismatch and a HLA-DRB1 and -DQB1 match. **Chapter 4 and 6** address the value of the MHC sequence similarity algorithms HLAMatchmaker or HistoCheck for the prediction of the CTLp assay outcome. **Chapter 5** is a more basic analysis on the relation between MHC class I sequence differences leading to a plausible description of acceptable mismatches that do not lead to T cell alloreactivity in vitro. **Chapter 7** describes the clinical relevance of these newly found permissible MHC in SCT with single MHC class I mismatched grafts. Finally, **chapter 8** is to put this all into perspective.

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